Figure 1a

MDSEAFQSARDFLDMNFQSLAMKHMDLKQMELDTAAAKVDELTKQLESLWSDSPAPPGPQAGP
PSRPPRYSSSSIPEPFGSRGSPRKAATDGADTPFGRSESAPTLHPYSPLSPKGRPSSPRTPLYLQPDAY
GSLDRATSPRPRAFDGAGSSLGRAPSPRPGPGPLRQQGPPTPFDFLGRAGSPRGSPLAEGPQAFFPE
RGPSPRPPATAYDAPASAFGSSLLGSGGSAFAPPLRAQDDLTLRRRPPKAWNESDLDVAYEKKPSQ
TASYERLDVFARPASPSLQLLPWRESSLDGLGGTGKDNLTSATLPRNYKVSPLASDRRSDAGSYRR
SLGSAGPSGTLPRSWQPVSRIPMPPSSPQPRGAPRQRPIPLSMIFKLQNAFWEHGASRAMLPGSPLF
TRAPPPKLQPQPQPQPQPQSQPQPQLPPQPQTQPQTPTPAPQHPQQTWPPVNEGPPKPPTELEPEPEI
EGLLTPVLEAGDVDEGPVARPLSPTRLQPALPPEAQSVPELEEVARVLAEIPRPLKRRGSMEQAPA
VALPPTHKKQYQQIISRLFHRHGGPGPGGPEPELSPITEGSEARAGPPAPAPPAPIPPPAPSQSSPPEQ
PQSMEMRSVLRKAGSPRKARRARLNPLVLLLDAALTGELEVVQQAVKEMNDPSQPNEEGITALH
NAICGANYSIVDFLITAGANVNSPDSHGWTPLHCAASCNDTVICMALVQHGAAIFATTLSDGATAF
EKCDPYREGYADCATYLADVEQSMGLMNSGAVYALWDYSAEFGDELSFREGESVTVLRRDGPEE
TDWWWAALHGQEGYVPRNYFGLFPRVKPQRSKV*

Figure 1b

CCACGCGTCCGGGAAGCCCCCAGGTGCCAGGATCTGCCCGGATCCGCGCCCGCTCCGGCCGG
CACCATGGACAGCGAGGCATTCCAGAGCGCGCGGGACTTTCTGGACATGAACTTCCAGTCGCT
GGCCATGAAACACATGGATCTGAAGCAGATGGAGCTGGACACGGCGGCGGCCAAGGTGGATG
AACTGACCAAGCAGCTGGAGTCGCTGTGGTCAGACTCTCCCGCGCCTCCTGGCCCGCAGGCCG
GACCCCCTTCTAGGCCGCCCCGGTACAGCTCCAGCTCGATCCCTGAGCCCTTCGGCAGCCGAG
GGTCCCCCCGGAAGGCGGCCACCGACGGCGCAGACACCCCGTTCGGACGATCAGAGAGTGCC
CCAACCCTACACCCCTACAGCCCGCTGTCCCCCAAGGGACGGCCGTCGTCGCCGCGCACCCCG
CTCTACCTGCAGCCGGACGCCTACGGCAGCCTGGACCGCGCGACCTCGCCCCGGCCCCGCGCC
TTCGATGGCGCAGGCAGCTCCCTCGGCCGTGCGCCCTCCCCGCGGCCCGGGCCAGGCCCGCTC
CGCCAGCAGGGTCCCCCCACGCCTTTCGACTTCCTGGGCCGCGCAGGCTCCCCCCGCGGCAGC
CCCCTGGCGGAGGGGCCCCAGGCCTTCTTCCCCGAGCGTGGGCCGTCACCGCGCCCCCCTGCC
ACAGCCTACGACGCGCCAGCGTCCGCCTTCGGGAGCTCCCTGCTAGGCTCCGGCGGCAGCGCA
TTCGCCCCGCCTCTGCGCGCGCAAGACGACCTGACGCTGCGCCGGCGGCCTCCGAAAGCCTGG
AACGAGTCTGACCTGGACGTGGCGTACGAGAAGAAGCCTTCGCAGACAGCGAGCTATGAACG
CCTGGACGTCTTCGCAAGGCCTGCCTCGCCGAGCCTGCAGCTGTTGCCTTGGAGGGAGAGCAG
CCTGGATGGACTGGGGGGCACCGGCAAGGACAACCTCACTAGCGCCACCCTGCCGCGCAATT
ACAAGGTCTCTCCTCTGGCCAGCGACCGGCGTTCAGACGCGGGCAGCTACCGGCGCTCGCTGG
GCTCCGCGGGGCCGTCGGGCACTTTGCCTCGCAGCTGGCAGCCCGTCAGCCGCATCCCCATGC
CCCCCTCCAGCCCCCAGCCCCGCGGGGCCCCGCGCCAGCGTCCCATCCCCCTCAGCATGATCT
TCAAGCTGCAGAACGCCTTCTGGGAGCACGGGGCCAGCCGCGCCATGCTCCCTGGGTCCCCCC
TCTTCACCCGAGCACCCCCGCCTAAGCTGCAGCCCCAACCACAACCACAGCCCCAGCCACAAT
CACAACCACAGCCCCAGCTGCCCCCACAGCCCCAGACCCAACCCCAAACCCCTACCCCAGCCC
CCCAGCATCCCCAACAGACATGGCCCCCTGTGAACGAAGGACCCCCCAAACCCCCCACCGAG
CTGGAGCCTGAGCCGGAGATAGAGGGGCTGCTGACACCAGTGCTGGAGGCTGGCGATGTGGA
TGAAGGCCCTGTAGCAAGGCCTCTCAGCCCCACGAGGCTGCAGCCAGCACTGCCACCGGAGG
CACAGTCGGTGCCCGAGCTGGAGGAGGTGGCACGGGTGTTGGCGGAAATTCCCCGGCCCCTC
AAACGCAGGGGCTCCATGGAGCAGGCCCCTGCTGTGGCCCTGCCCCCTACCCACAAGAAACA
GTACCAGCAGATCATCAGCCGCCTCTTCCATCGTCATGGGGGGCCAGGGCCCGGGGGGCCGG
AGCCAGAGCTGTCCCCCATCACTGAGGGATCTGAGGCCAGGGCAGGGCCCCCTGCTCCTGCCC
CACCAGCTCCCATTCCACCCCCGGCCCCGTCCCAGAGCAGCCCACCAGAGCAGCCGCAGAGC
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CAACCCTCTGGTGCTCCTCCTGGACGCGGCGCTGACCGGGGAGCTGGAGGTGGTGCAGCAGG
CGGTGAAGGAGATGAACGACCCGAGCCAGCCCAACGAGGAGGGCATCACTGCCTTGCACAAC
GCCATCTGCGGCGCCAACTACTCTATCGTGGATTTCCTCATCACCGCGGGTGCCAATGTCAAC
TCCCCCGACAGCCACGGCTGGACACCCTTGCACTGCGCGGCGTCGTGCAACGACACAGTCATC
TGCATGGCGCTGGTGCAGCACGGCGCTGCAATCTTCGCCACCACGCTCAGCGACGGCGCCACC
GCCTTCGAGAAGTGCGACCCTTACCGCGAGGGTTATGCTGACTGCGCCACCTACCTGGCAGAC
GTCGAGCAGAGTATGGGGCTGATGAACAGCGGGGCAGTGTACGCTCTCTGGGACTACAGCGC

CGAGTTCGGGGACGAGCTGTCCTTCCGCGAGGGCGAGTCGGTCACCGTGCTGCGGAGGGACG
GGCCGGAGGAGACCGACTGGTGGTGGGCCGCGCTGCACGGCCAGGAGGGCTACGTGCCGCGG
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GAGGTTTCTGAGGCTGACAGAAACAAGCATTCCTGCCTTCCCTCCAGACCTCTCCCTCTGTTTT
TTGCTGCCTTTATCTGCACCCCTCACCCTGCTGGTGGTGGTCCTTGCCACCGGTTCTCTGTTCTC
CTGGAAGTCCAGGGAAGAAGGAGGGCCCCAGCCTTAAATTTAGTAATCTGCCTTAGCCTTGGG
AGGTCTGGGAAGGGCTGGAAATCACTGGGGACAGGAAACCACTTCCTTTTGCCAAATCAGAT
CCCGTCCAAAGTGCCTCCCATGCCTACCACCATCATCACATCCCCCAGCAAGCCAGCCACCTG
CCCAGCCGGGCCTGGGATGGGCCACCACACCACTGGATATTCCTGGGAGTCACTGCTGACACC
ATCTCTCCCAGCAGTCTTGGGGTCTGGGTGGGAAACATTGGTCTCTACCAGGATCCCTGCCCC
ACCTCTCCCCAATTAAGTGCCTTCACACAGCTCTGGTTTAATGTTTATAAACAAAATAGAGAA
ACTTTCCTTATAAATAAAAGTAGTTTGCACAGAAAAAAAAAAAAAAA

Figure 2a

MWMKDPVARPLSPTRLQPALPPEAQSVPELEEVARVLAEIPRPLKRRGSMEQAPAVA
LPPTHKKQYQQIISRLFHRHGGPGPGGRSQSCPPSLRDLRPGQGPLLLPHQLPFHRPAP
SQSSPPEQPQSMEMRSVLRKAGSPRKARRARLNPLVLLLDAALTGELEVVQQAVKE
MNDPSQPNEEGITALHNAICGANYSIVDFLITAGANVNSPDSHGWTPLHCAASCNDT
VICMALVQHGAAIFATTLSDGATAFEKCDPYREGYADCATYLADVEQSMGLMNSGA
VYALWDYSAEFGDELSFREGESVTVLRRDGPEETDWWWAALHGQEGYVPRNYFGL
FPRVKPQRSKV

Figure 2b

GCGGCCGCGTCGACCCGGCGTTCAGACGCGGGCAGCTACCGGCGCTCGCTGGGTCCGCGGGGCCGTC
GGGCACTTTGCCTCGCAGCTGGCAGCCCGTCAGCCGCATCCCATGCCCCCCTCCAGCCCCCAGCCCC
GCGGGGCCCCGCGCCAGCGTCCCATCCCCCTCAGCATGATCTTCAAGCTGCAGAACGCCTTCTGGGA
GCACGGGGCCAGCCGCG CCATGCTCCCTGGGTCCCCCCTCTTCACCCGAGCACCCCCGCCTAAGCTG
CAGCCCCAACCACAACCACAGCCCCAGCCACAATCACAACCACAGCCCCAGCTGCCCCAACAGCCCC
AGACCCAACCCCAAACCCCTACCCCAGCCTCCCACATCCGCATCCCCAACAGACATGGCCCCCTGTG
AACGAAGGACCCCCCAAACCCCCCACCGAGCTGGAGCCTGAGCCGGAGATAGAGGGGCTGCTGACA
CCAGTGCTGGAGGCTGGCGATGTGGATGAAGGACCCTGTAGCAAGGCCTCTCAGCCCCACGAGGCTG
CAGCCAGCACTGCCACCGGAGGCACAGTCGGTGCCCGAGCTGGAGGAGGTGGCACGGGTGTTGGCG
GAAATTCCCCGGCCCCTCAAACGCAGGGGCTCCATGGAGCAGGCCCCTGCTGTGGCCCTGCCCCCTA
CCCACAAGAAACAGTACCAGCAGATCATCAGCCGCCTCTTCCATCGTCATGGGGGGCCAGGGCCCGG
GGGGCGGAGCCAGAGCTGTCCCCCATCACTGAGGGATCTGAGGCCAGGGCAGGGCCCCCTGCTCCTG
CCCCAC CAGCTCCCATTCCACCGCCCGGCCCCGTCCCAGAGCAGCCCACCAGAGCAGCCGCAGAGC
ATGGAGATGCGCTCTGTGCTGCGGAAGGCGGGCTCCCCGCGCAAGGCCCGCCGCGCGCGCCTCAACC
CTCTGGTGCTCCTCCTGGACGCGGCGCTGACCGGGGAGCTGGAGGTGGTGCAGCAGGCGGTGAAGG
AGATGAACGACCCGAGCCAGCCCAACGAGGAGGGCATCACTGCCTTGCACAACGCCATCTGCGGCG
CCAACTACTCTATCGTGGATTTCCTCATCACCGCGGGTGCCAATGTCAACTCCCCCGACAGCCACGGC
TGGACACCCTTGCACTGCGCGGCGTCGTGCAACGACACAGTCATCTGCATGGCGCTGGTGCAGCACG
GCGCTG CAATCTTCGC CACCACGCTC AGCGACGGCG CCACCGCCTTCGAGAAGTGCGACCCTTACC
GCGAGGGTTATGCTGACTGCGCCACCTACCTGGCAGACGTCGAGCAGAGTATGGGGCTGATGAACA
GCGGGGCAGTGTACGCTCTCTGGGACTACAGCGCCGAGTTCGGGGACGAGCTGTCCTTCCGCGAGGG
CGAGTCGGTCACCGTGCTGCGGAGGGACGGGCCGGAGGAGACCGACTGGTGGTGGGCCGCGCTGCA
CGGCCAGGAGGGCTACGTGCCGCGGAACTACTTCGGGCTGTTCCCCAGGGTGAAGCCTCAAAGGAGT
AAAGTCTAGCAGGATAGAAGGAGGTTTCTGAGGCTGACAGAAACAAGCATTCCTGCCTTCCCTCCAG
ACCTCTC CCTCTGTTTTTTGCTGCCTT TATCTGCACC CCTCACCCTG CTGGTGGTGG TCCTTGCCAC
CGGTTCTCTGTTCTCCTGGAAGTCCAGGGAAGAAGGAGGGCCCCAGCCTTAAATTTAGTAATCTGCC
TTAGCCTTGGGAGGTCTGGGAAGGGCTGGAAATCACTGGGGACAGGAAACCACTTCCTTTTGCCAAA
TCAGAT CCCGTCCAAA GTGCCTCCCA TGCCTACCAC CATCATCACA TCCCCCAGCAAGCCAGCCAC
CTGCCCAGCCGGGCCTGGGATGGGCCACCACACCACTGGATATTCCTGGGAGTCACTGCTGACACCA
TCTCTCCCAGCAGTCTTGGGGTCTGGGTGGGAAACATTGGTCTCTACCAGGATCCCTGCCCCACCTCT
CCCCA ATTAAGTGCC TTCACACAGC ACTGGTTTAATGTTTATAAA CAAAATAGAG AAACTGGTTT
AATGTTTATA AACAAAATAG AGAAACTTTCGCTTATAAAT AAAAGTAGTT TGCACAGAAA
TGAAAAAAAA AAAAAAAAAA AAAAAA

Figure 3.1

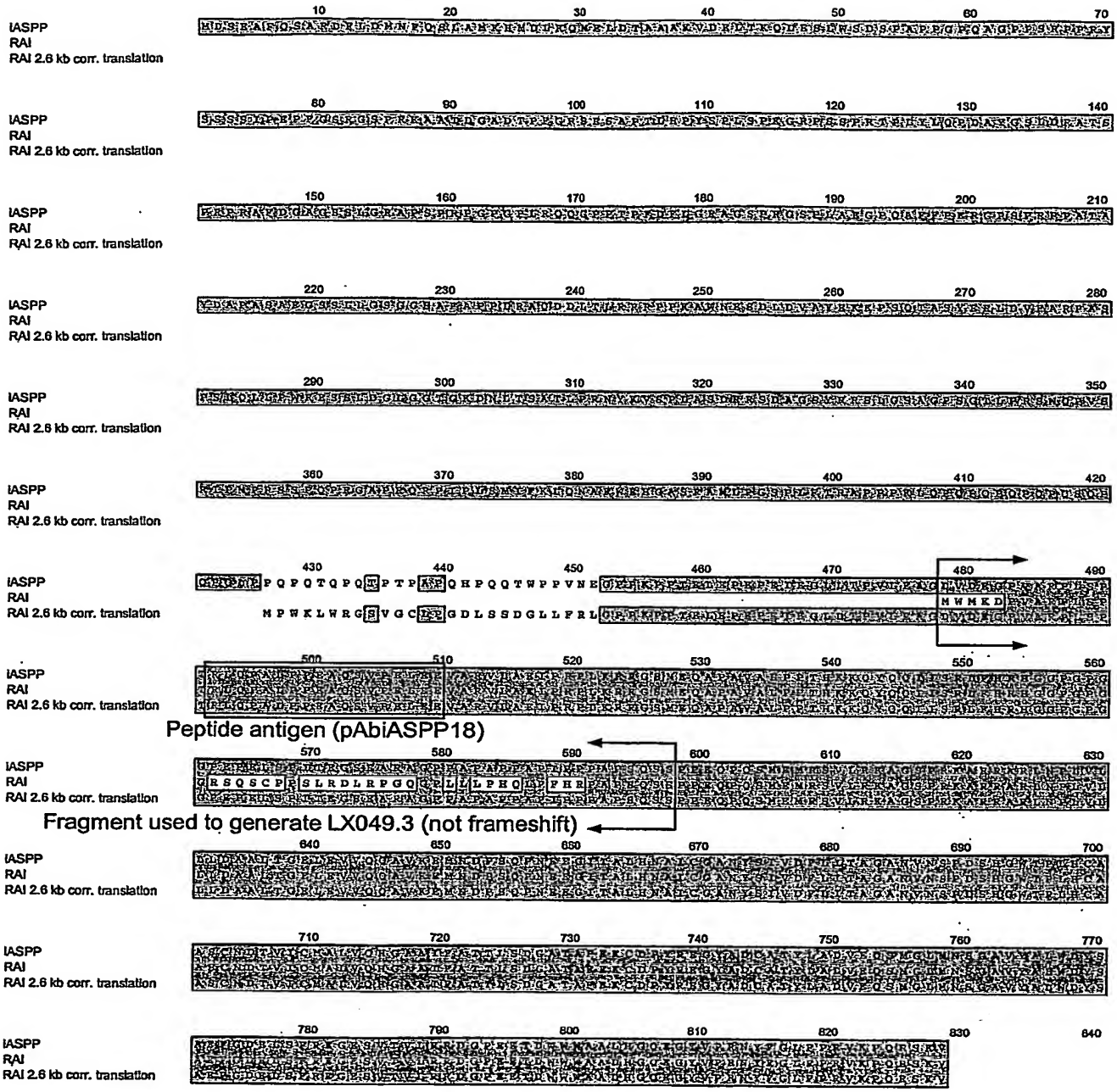

lASPP
RAI
RAI 2.6 kb corr. translation
Peptide antigen (pAbiASPP18)
Fragment used to generate LX049.3 (not frameshift)

Figure 3.2

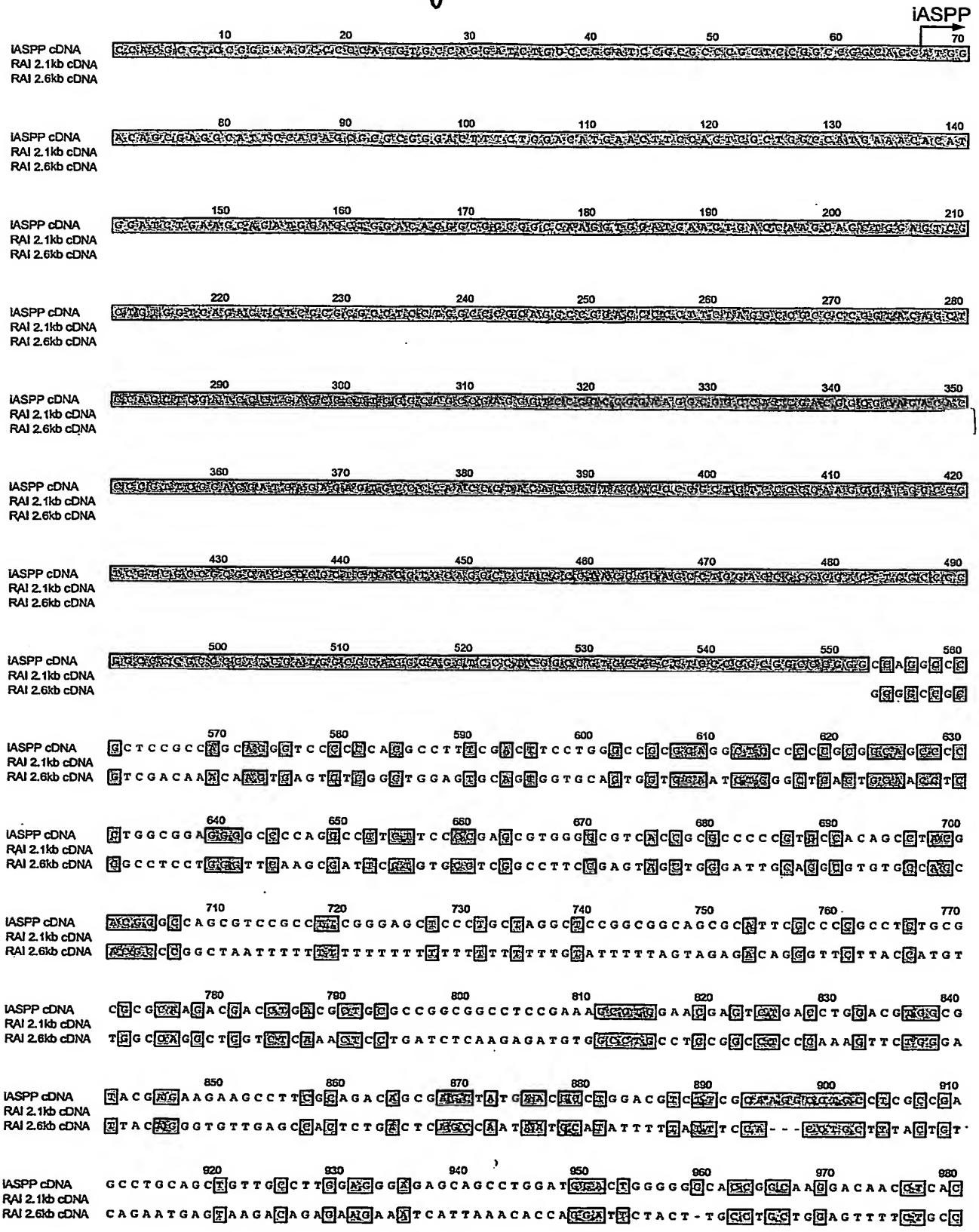


Figure 3.3

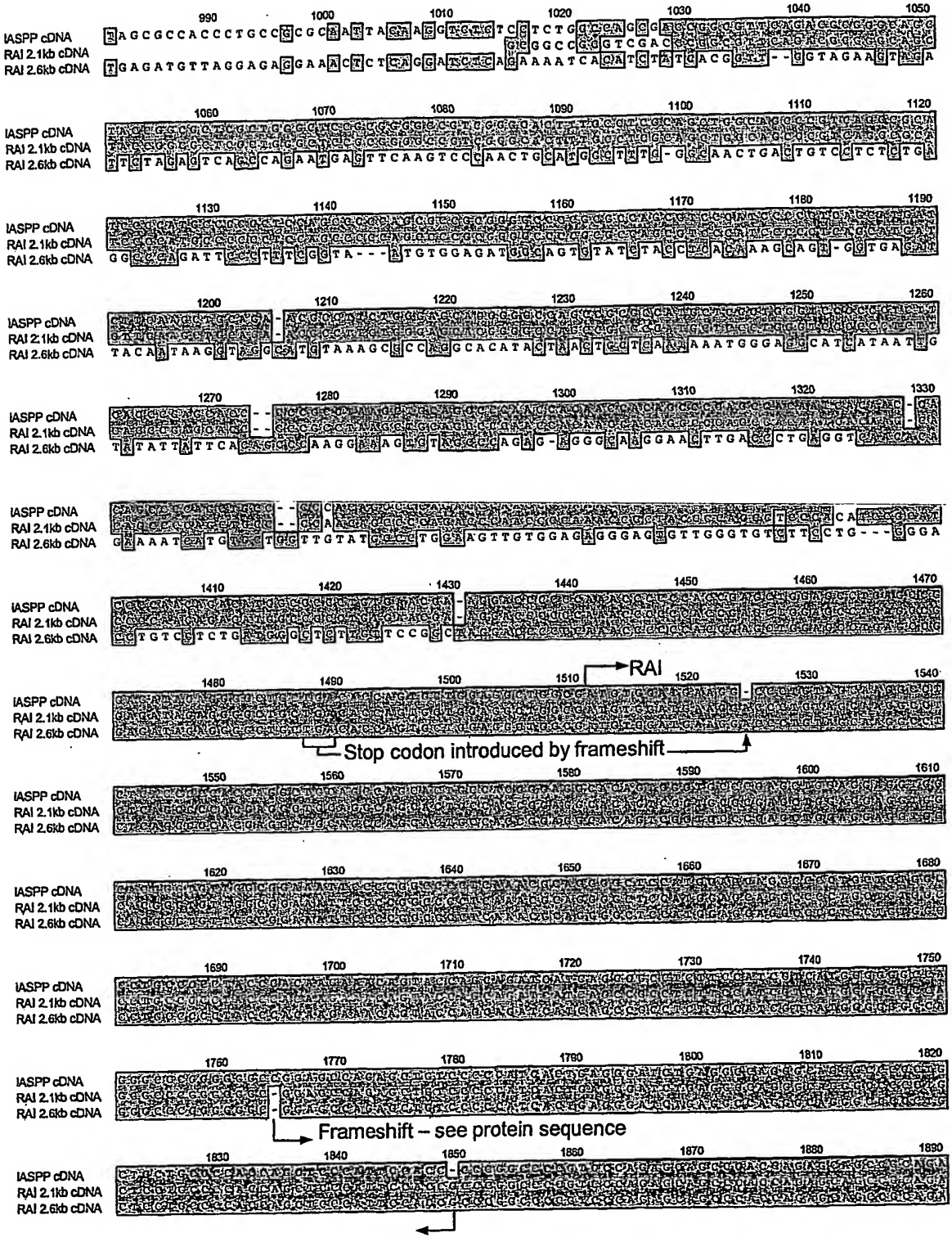

IASPP cDNA
RAI 2.1kb cDNA
RAI 2.6kb cDNA
RAI
Stop codon introduced by frameshift
Frameshift – see protein sequence

Figure 3.4

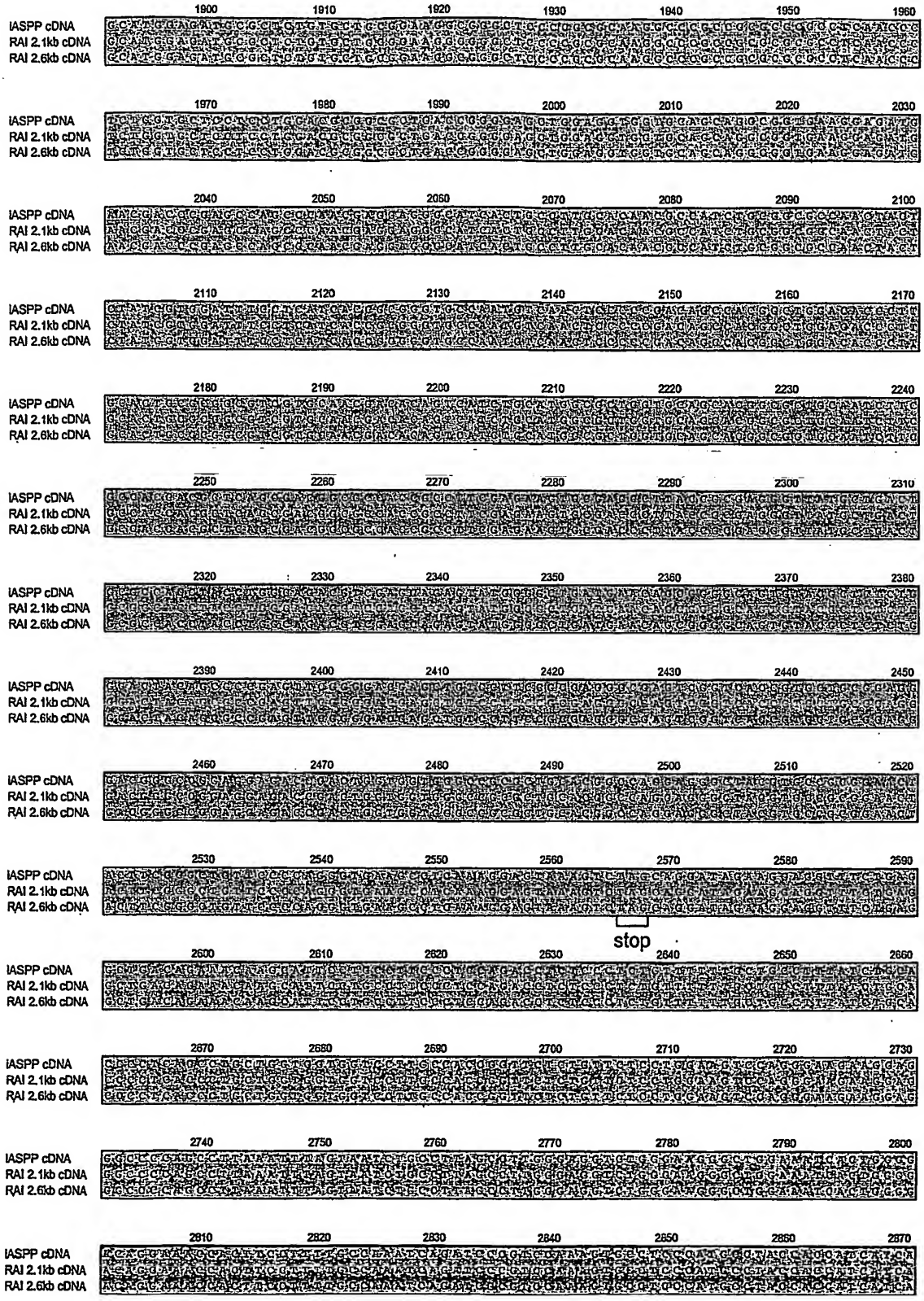

1900 1910 1920 1930 1940 1950 1960
iASPP cDNA
RAI 2.1kb cDNA
RAI 2.6kb cDNA
1970 1980 1990 2000 2010 2020 2030
2040 2050 2060 2070 2080 2090 2100
2110 2120 2130 2140 2150 2160 2170
2180 2190 2200 2210 2220 2230 2240
2250 2260 2270 2280 2290 2300 2310
2320 2330 2340 2350 2360 2370 2380
2390 2400 2410 2420 2430 2440 2450
2460 2470 2480 2490 2500 2510 2520
2530 2540 2550 2560 2570 2580 2590
stop
2600 2610 2620 2630 2640 2650 2660
2670 2680 2690 2700 2710 2720 2730
2740 2750 2760 2770 2780 2790 2800
2810 2820 2830 2840 2850 2860 2870

Figure 3.5

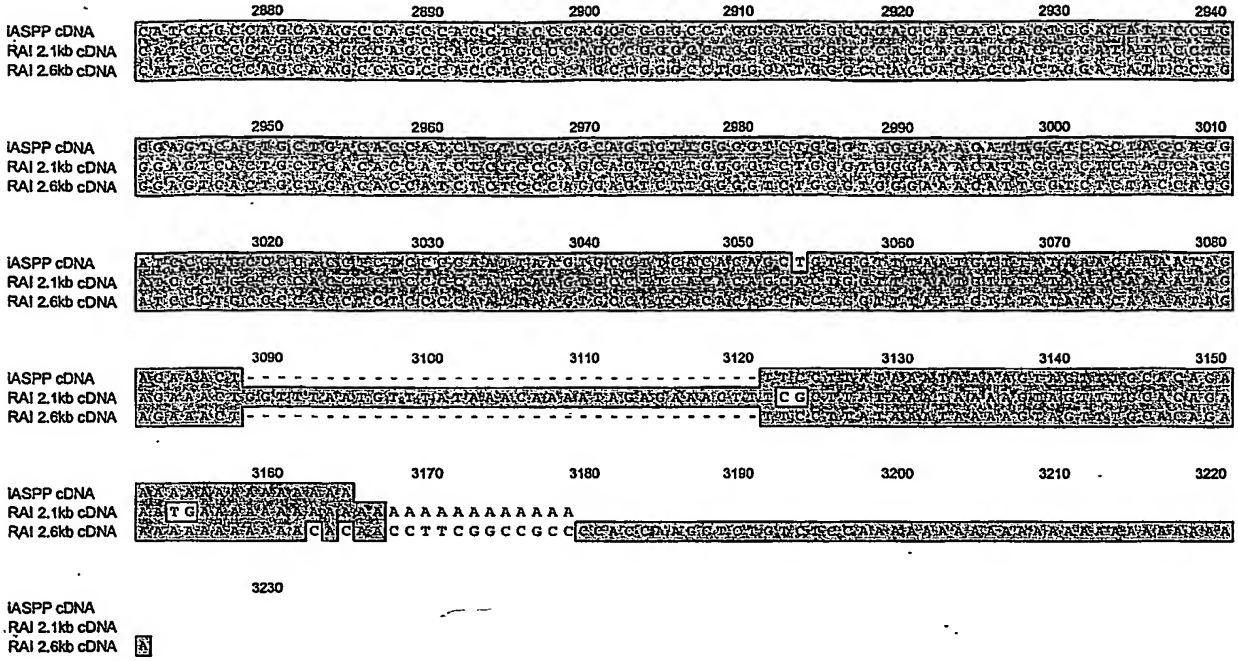

2880
2890
2900
2910
2920
2930
2940
2950
2960
2970
2980
2990
3000
3010
3020
3030
3040
3050
3060
3070
3080
3090
3100
3110
3120
3130
3140
3150
3160
3170
3180
3190
3200
3210
3220
3230
IASPP cDNA
RAI 2.1kb cDNA
RAI 2.6kb cDNA

Figure 4a

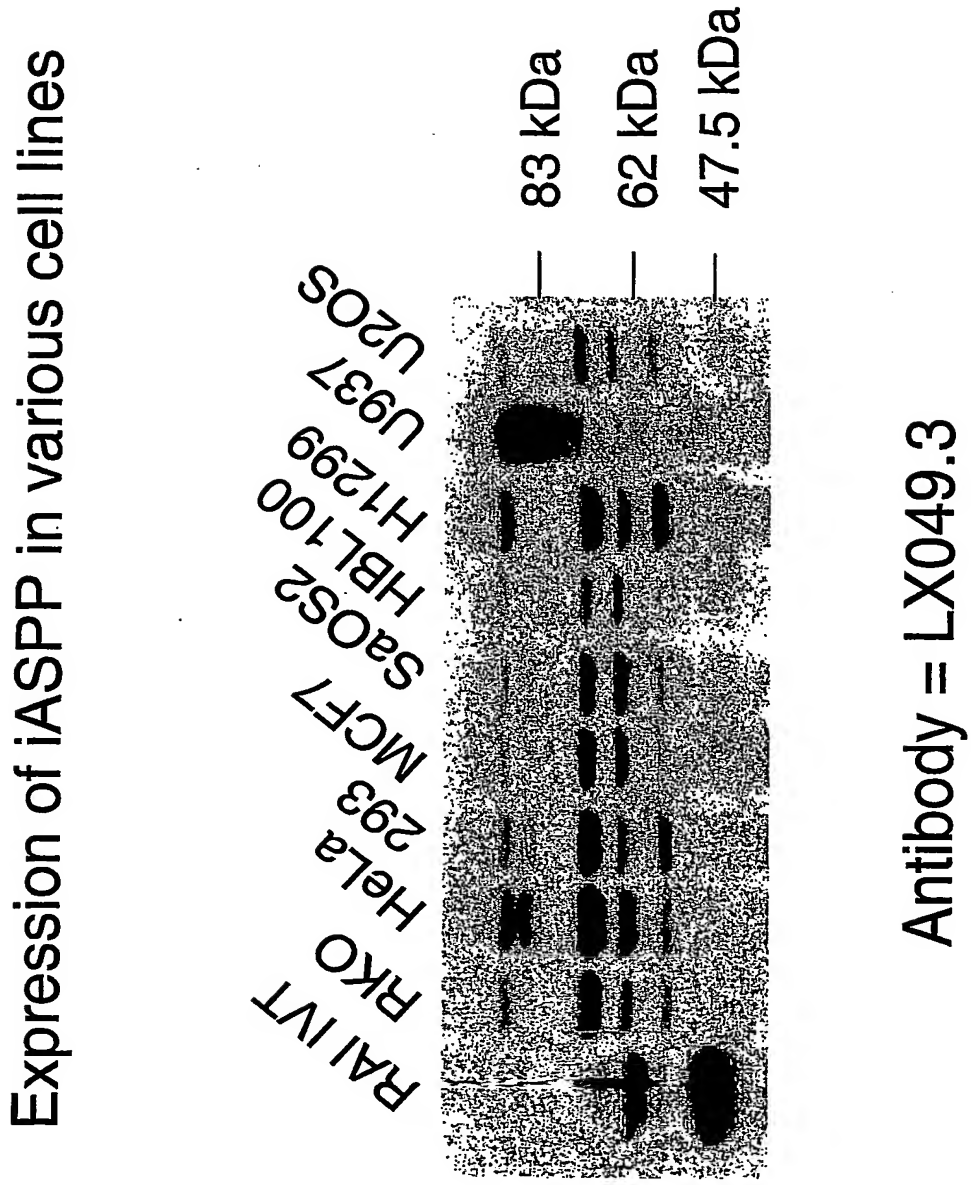

Expression of iASPP in various cell lines
83 kDa
62 kDa
47.5 kDa
RAI IVT
RKO
HeLa
293
MCF7
SaOS2
HBL100
H1299
U937
U2OS
Antibody = LX049.3

Figure 4B

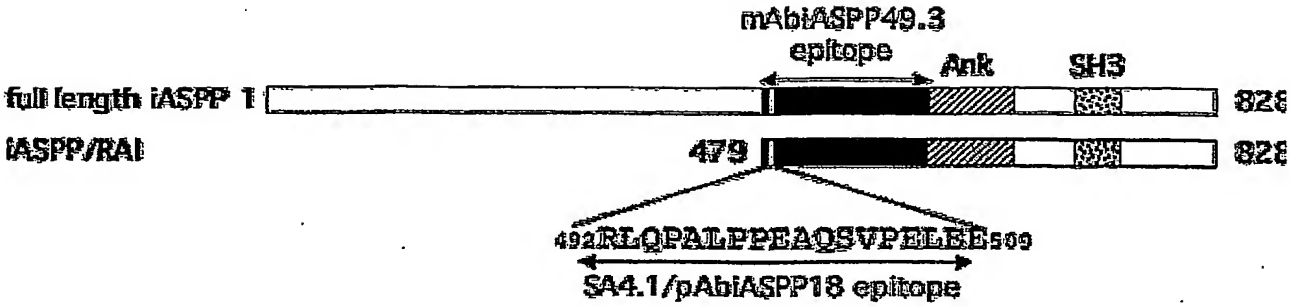


Figure 4C

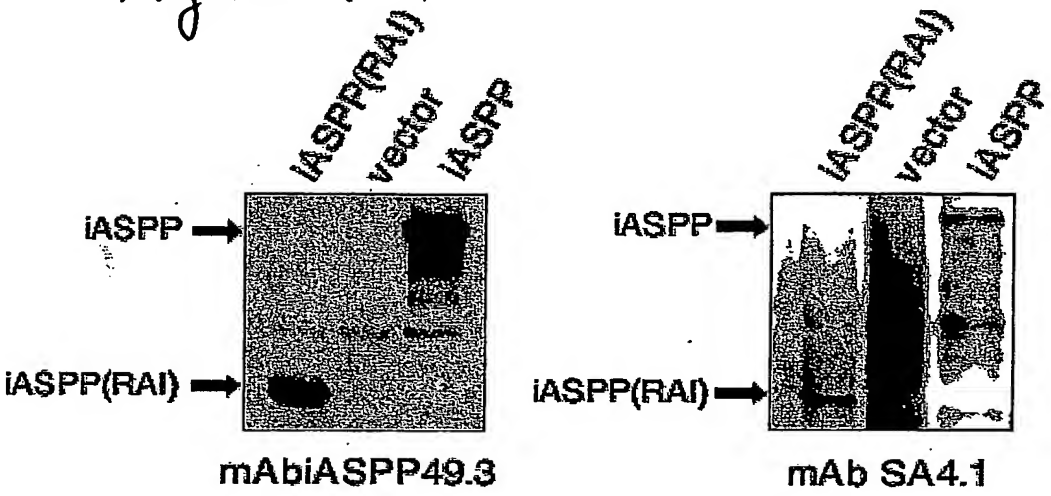


Figure 4D

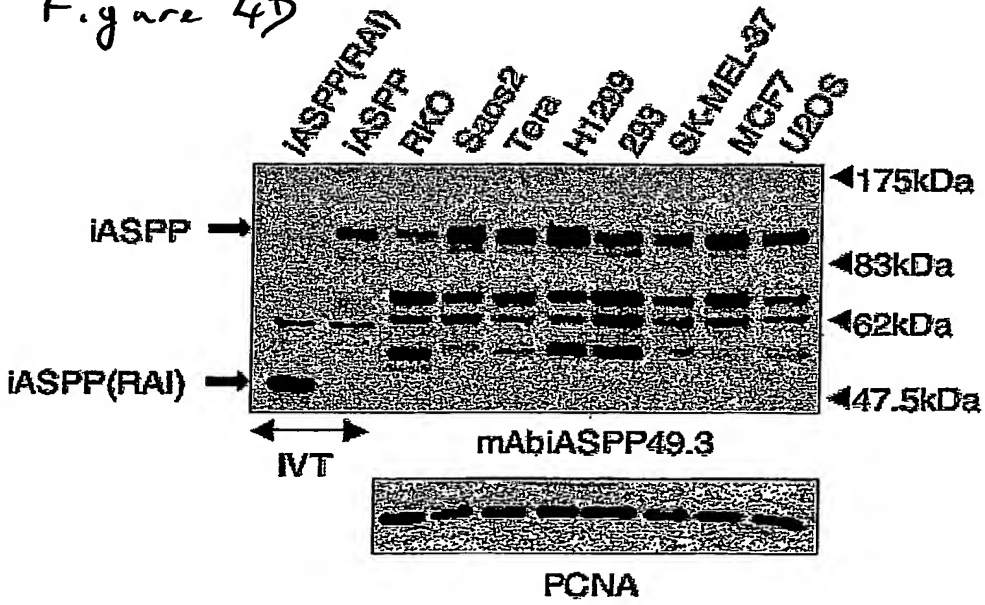


Figure 5

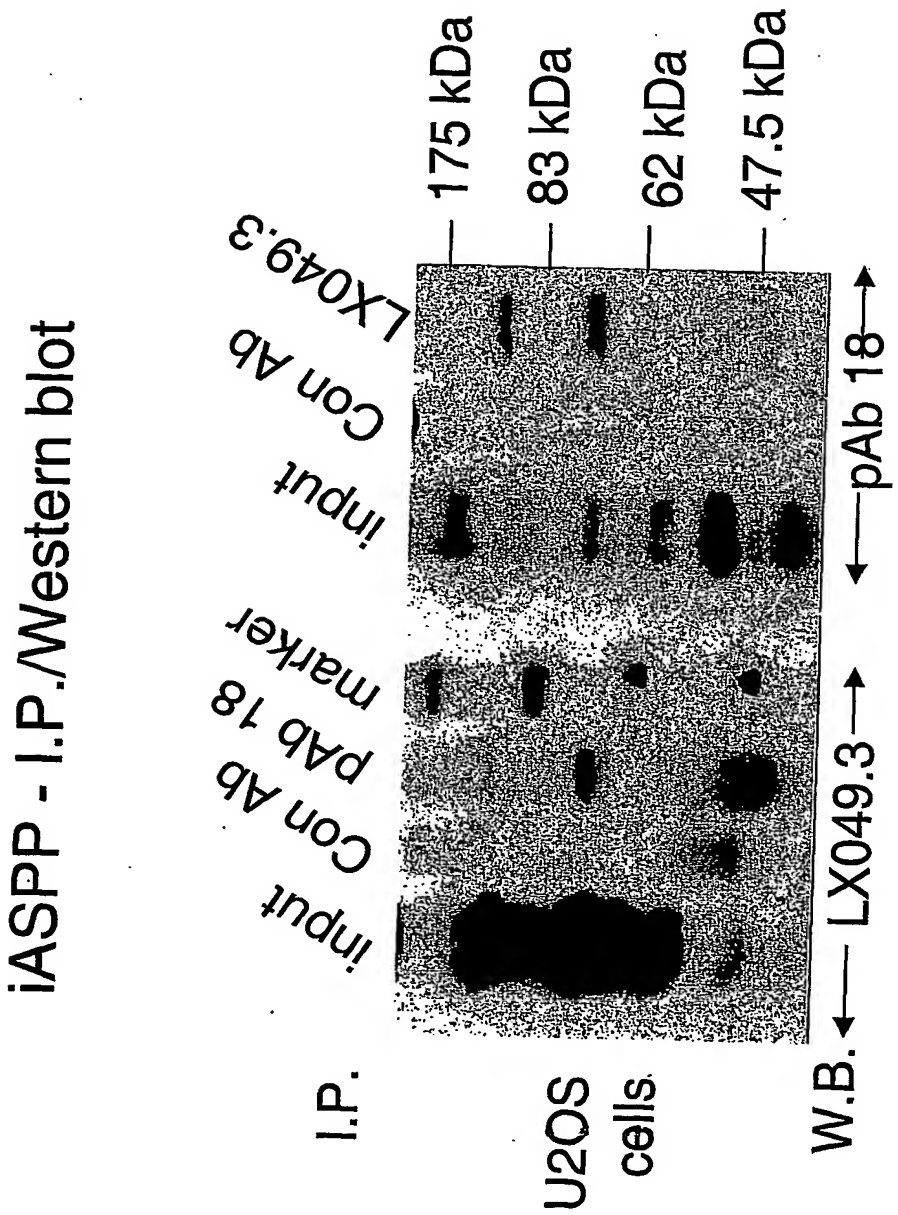

iASPP - I.P./Western blot
I.P.
input
Con Ab
LX049.3
input
Con Ab
PAb 18
marker
U2OS cells
W.B.
LX049.3
pAb 18
175 kDa
83 kDa
62 kDa
47.5 kDa

Figure 6a

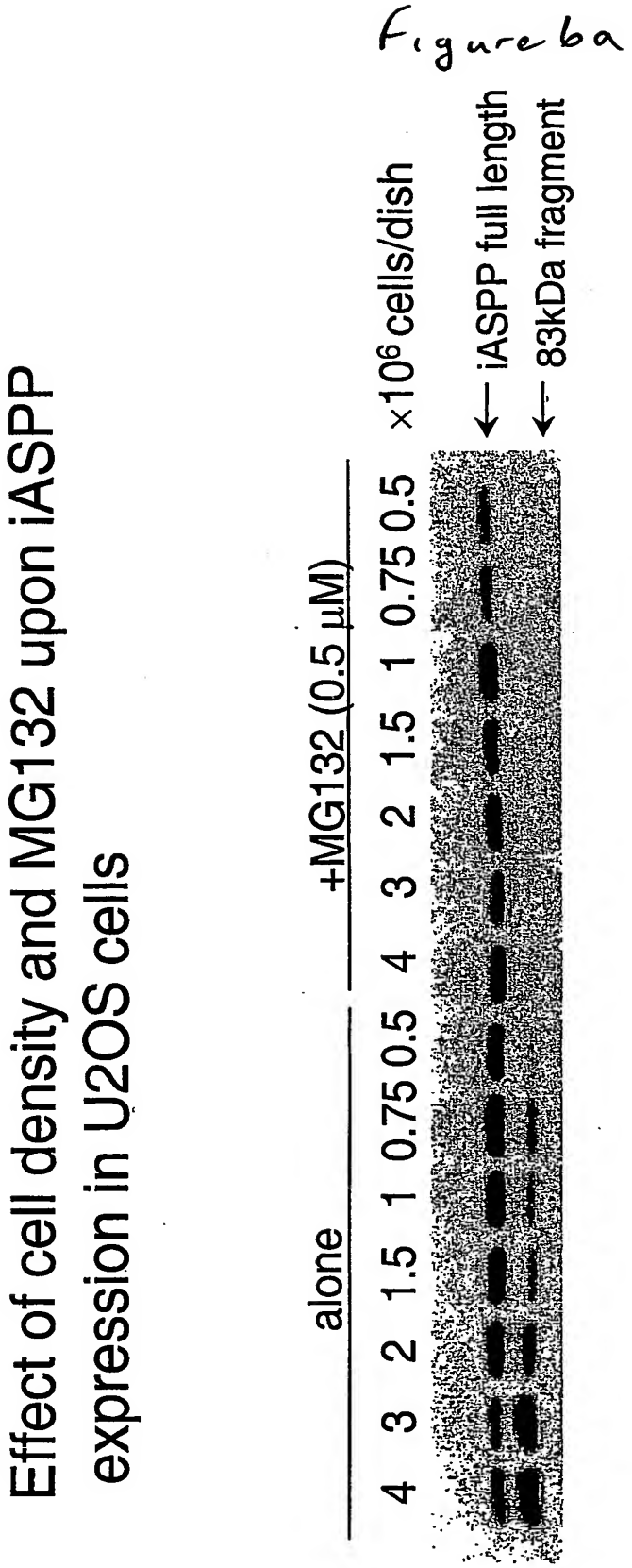

Effect of cell density and MG132 upon iASPP expression in U2OS cells
alone
+MG132 (0.5 μM)
4 3 2 1.5 1 0.75 0.5 4 3 2 1.5 1 0.75 0.5
×10^6 cells/dish
← iASPP full length
← 83kDa fragment

Figure 6b

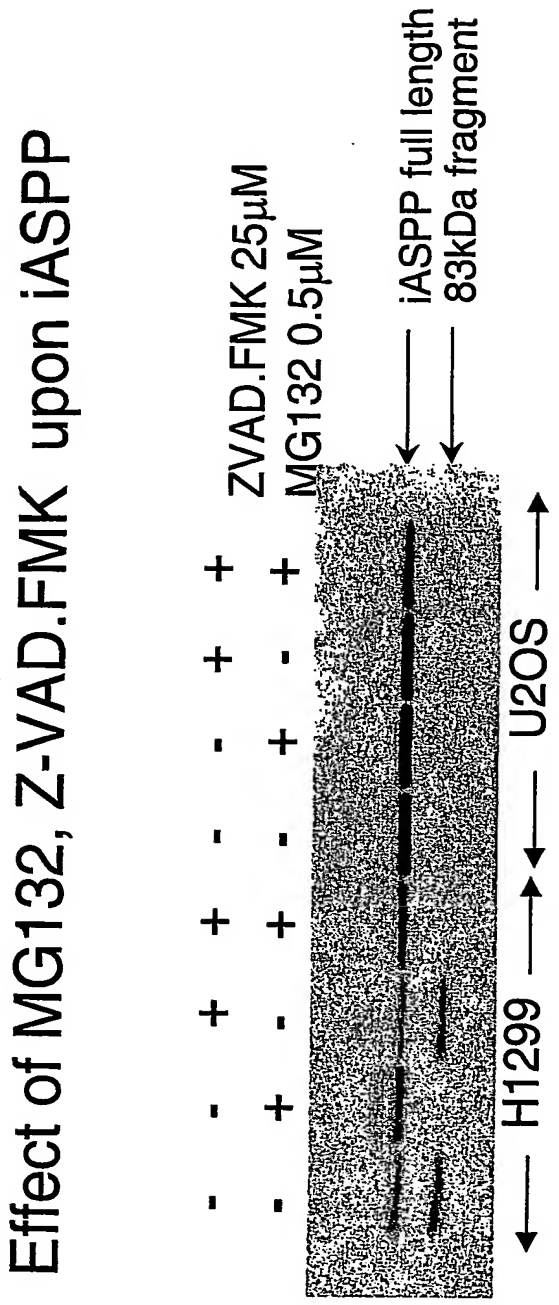

Effect of MG132, Z-VAD.FMK upon iASPP
ZVAD.FMK 25μM
MG132 0.5μM
iASPP full length
83kDa fragment
U2OS
H1299

Figure 7

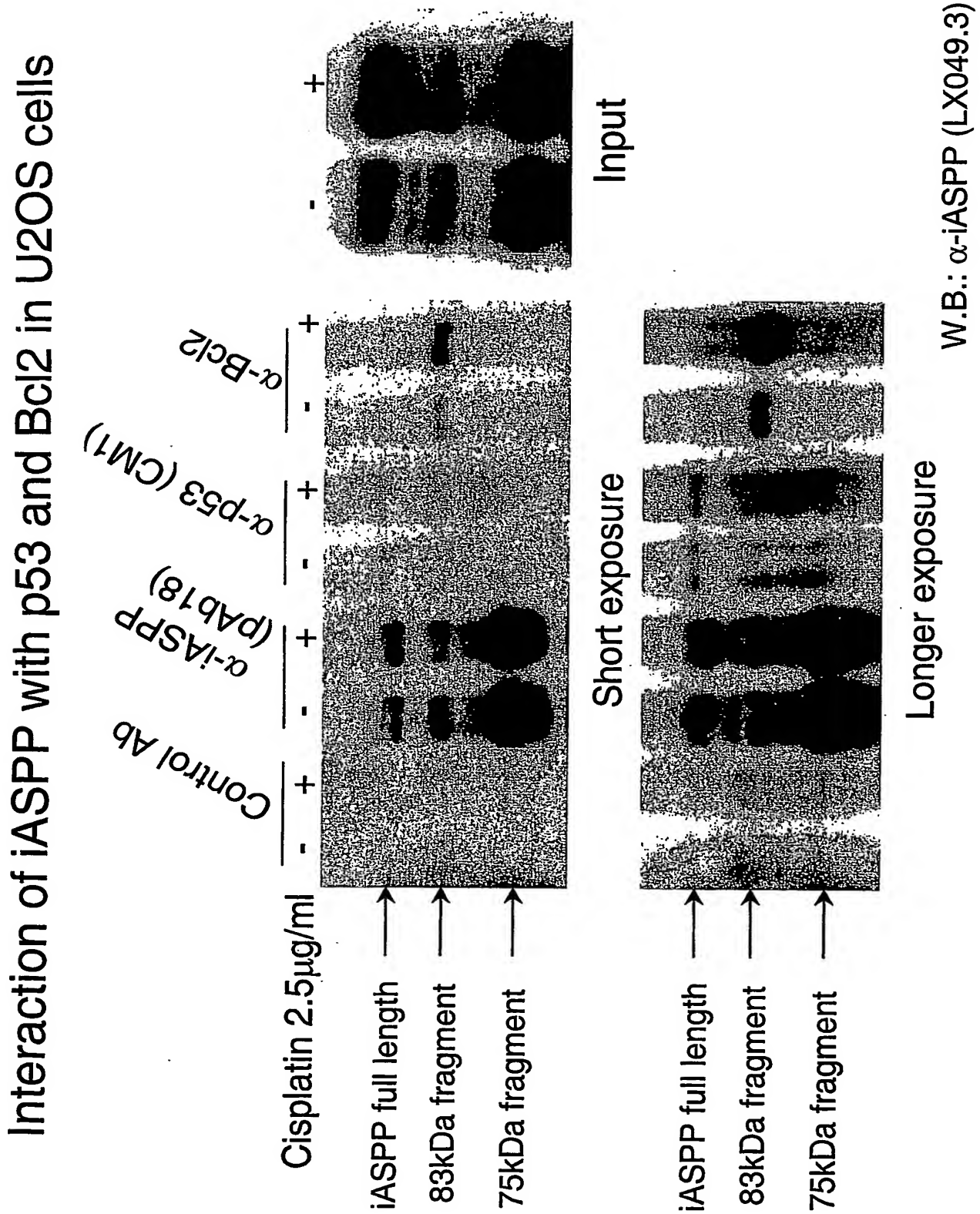

Interaction of iASPP with p53 and Bcl2 in U2OS cells
Control Ab
α-iASPP (pAb18)
α-p53 (CM1)
α-Bcl2
Cisplatin 2.5μg/ml
iASPP full length
83kDa fragment
75kDa fragment
Input
Short exposure
Longer exposure
W.B.: α-iASPP (LX049.3)

Figure 8

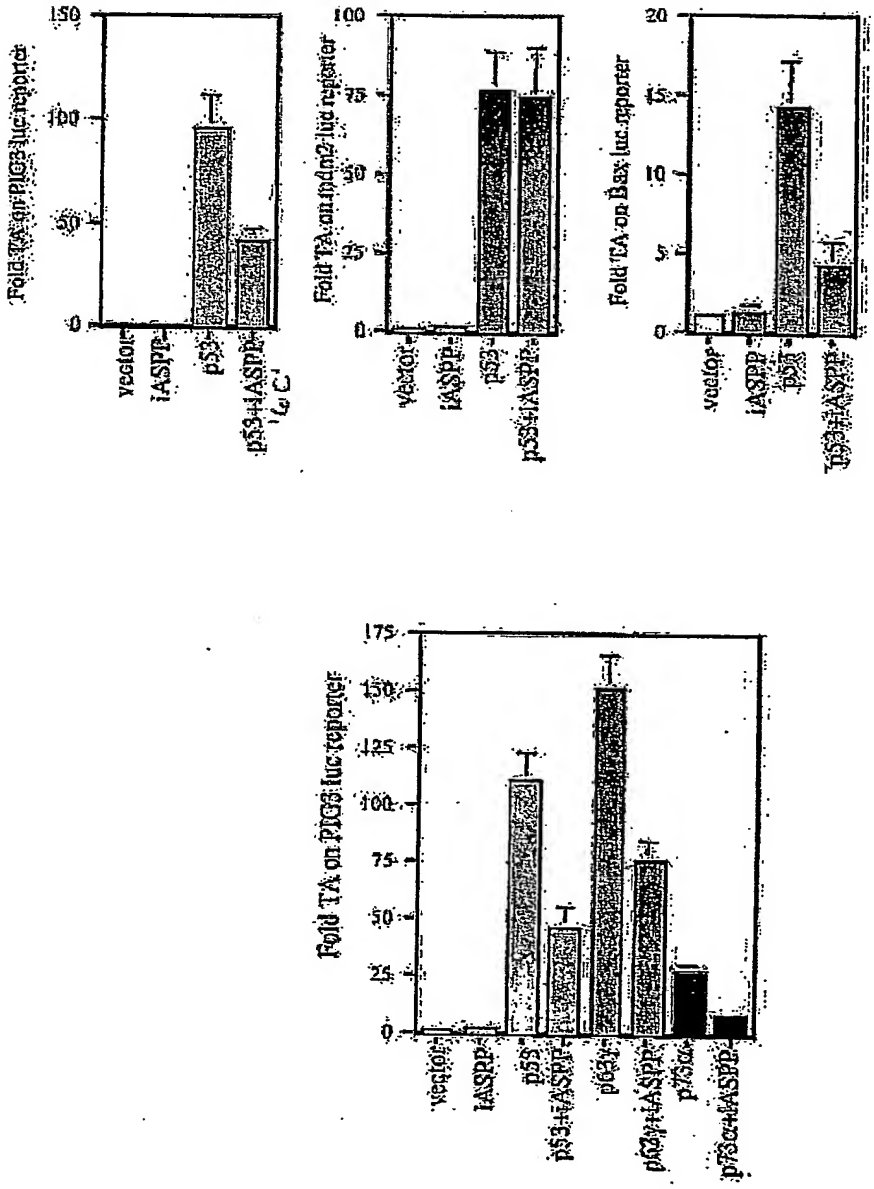
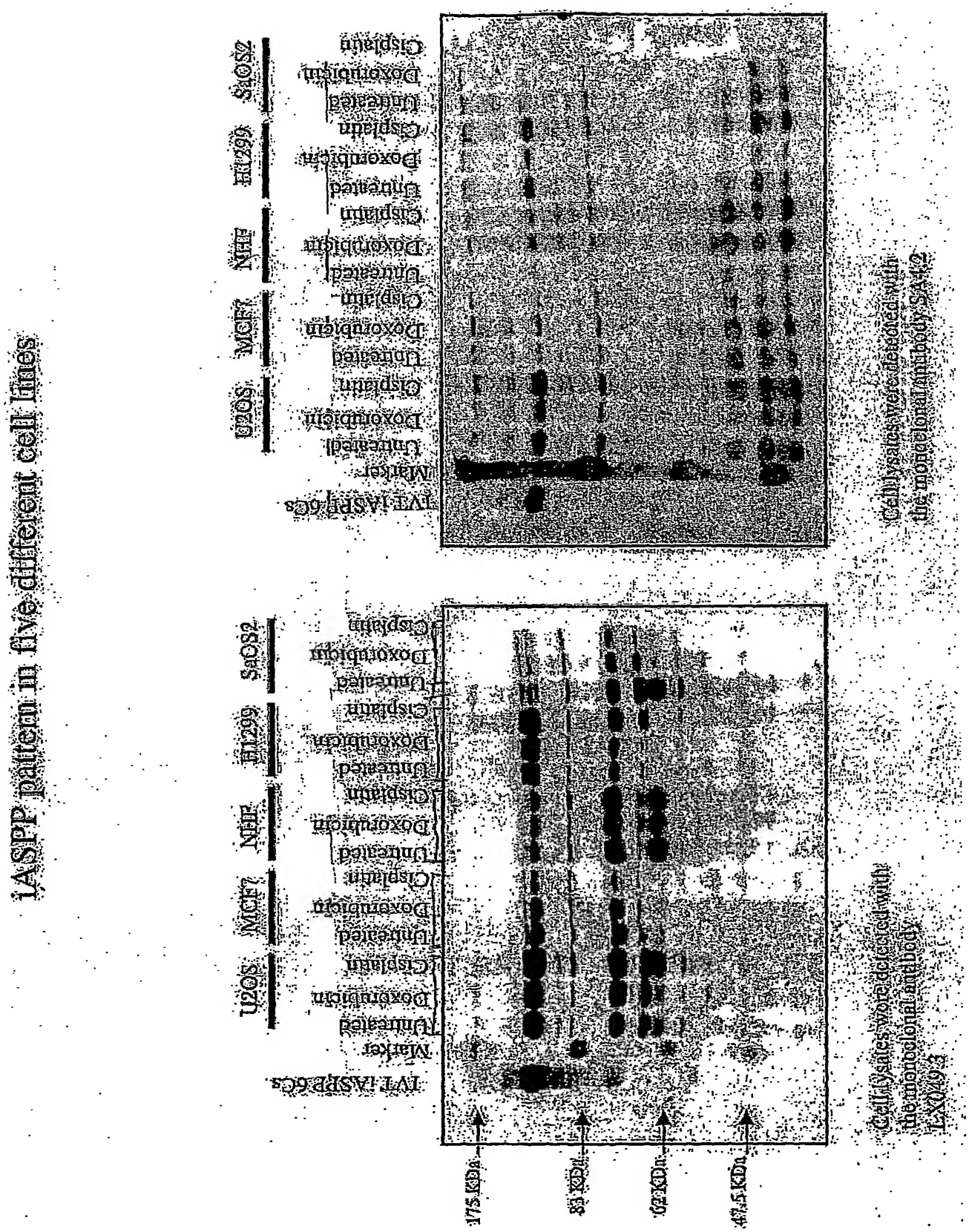

Fold TA on PIG3-luc reporter
150
100
50
0
vector
iASPP
p53
p53+iASPP
Fold TA on mdm2-luc reporter
100
75
50
25
0
vector
iASPP
p53
p53+iASPP
Fold TA on Bax-luc reporter
20
15
10
5
0
vector
iASPP
p53
p53+iASPP
Fold TA on PIG3-luc reporter
175
150
125
100
75
50
25
0
vector
iASPP
p53
p53+iASPP
p63γ
p63γ+iASPP
p73α
p73α+iASPP

Figure 9


iASPP pattern in five different cell lines
IVT iASPP 6Cs
Marker
Untreated
Doxorubicin
Cisplatin
Untreated
Doxorubicin
Cisplatin
Untreated
Doxorubicin
Cisplatin
Untreated
Doxorubicin
Cisplatin
U2OS
MCF7
NHF
H1299
SaOS2
175 KDa
83 KDa
62 KDa
47.5 KDa
Cell lysates were detected with the monoclonal antibody LX09.3
Cell lysates were detected with the monoclonal antibody SA4.2